INCOMPLETE ANTERIOR REGENERATION IN THE ABSENCE OF THE BRAIN IN LEPTOPLANA LITTORALIS.

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Previous experiments on the regeneration of planarians, more particularly of marine forms, seem to indicate that the presence of the cephalic ganglia is an important factor in regeneration. The following experiments were carried out to test the question further. The species used was a large marine planarian, *Leptoplana littoralis*, very abundant at Pacific Grove, Cal., found under wet stones just above the low-water mark.

Lillie[1] and Morgan[2] have observed in *Dendrocœlum*, that the power of regeneration is limited in posterior pieces, the regeneration anteriorly never being complete if more than about the anterior third of the worm is cut off. Schultz,[3] found in *Leptoplana* that anterior regeneration of a posterior piece cut at any level posterior to the cephalic ganglia is very slow and rarely if ever complete. Child[4] observed the same fact, and also in the case of lateral regeneration that "when they [the cephalic ganglia] are absent neither they nor the lateral part of the head removed are regenerated, though lateral regeneration of other parts may be more or less complete in the absence of the ganglia." In *L. littoralis* there is also a very marked difference in the rate and amount of regeneration of different kinds of pieces, and also in the behavior of the pieces.

Pieces of any size and shape, in which the cephalic ganglia remained, responded quickly to stimuli that affect the normal worm, and in general behaved like normal animals; moreover those that lived regenerated rapidly. Even small pieces (cut out

[1] Lillie, F. R., '01, "Notes on Regeneration and Regulation in Planarians," *Am. Journ. of Physiology*, Vol. XI., p. 129.

[2] Morgan, T. H.,'04, "Notes on Regeneration," BIOL. BULLETIN, Vol. VI., p. 159.

[3] Schultz, E., '02, "Aus dem Gebiete der Regeneration, II., Ueber die Regeneration bei Turbellarien," *Zeitsch. der wiss. Zool.*, 72 Bd., p. 1.

[4] Child, C. M.,'04, "Studies on Regulation, IV., Some Experimental Modifications of Form-regulation in Leptoplana," *Journ. of Exp. Zool.*, Vol. I., p. 95.

of the head with a straw), containing the eye-spots and cephalic ganglia and very little tissue besides, responded readily to light and touch, and crawled actively ; in all cases in which such pieces survived, they quickly regenerated tissue in every direction, and in less than two weeks presented the appearance of small worms with heads very large in proportion to their tails (Fig. A, 7, 8). Pieces without the cephalic ganglia, on the other hand, behaved differently. They were sluggish in their movements, responded slowly and did not even stay under water, but were often found dried up on the sides of the dish. Also, as Child and Schultz found in the forms which they studied, anterior regeneration took place only to a very limited extent. Pieces cut at any level posterior to the ganglia often healed in such a way as to apparently prevent the growth of new tissue. After the worm was cut, the sides stretched very much and actually met in front of the worm, bringing the two halves of the cut surface in contact, so that they grew together, the point where they met in the middle line being raised above the level of the surface on which the worm crawled. To avoid such a closure of the wound, worms were in some cases cut so that the anterior end was pointed, or else the pieces were kept flat in vaseline under water. Even so, scarcely any anterior regeneration took place. When the posterior end of the same piece was also cut off, it was rapidly regenerated, proving, as in *Drendrocœlum*, that growth in the anterior direction was not wanting because of a general lack of power to regenerate in the absence of the cephalic ganglia. The pieces were not kept long enough to prove that anterior regeneration never could occur, but, whatever the limit, the rate is excessively slow as compared with the rate in other directions or in other sorts of pieces. One worm which was supposedly cut behind the brain, completely regenerated the anterior end including ganglia and eye-spots. It is undoubtedly true that it was cut posterior to the eye-spots, but the probability is that part at least of the ganglia remained. That this would be possible can readily be seen from the figure of a horizontal section of a normal worm, showing the relative positions of ganglia and eye-spots.*

* As this paper goes to press, a new paper has appeared by C. M. Child, "Studies on Regulation, VI., The Relation Between the Central Nervous System and Regula-

So far then the results showed nothing different from what had already been observed in other forms, but the power to regenerate ganglia had been tested only in pieces from which the tissue anterior to the level of the ganglia had been cut away, and the question remained whether that power was entirely wanting under all conditions. The ganglia were accordingly removed in a number of ways, all differing from a cut straight across the worm in that some of the tissue anterior to the level of the ganglia in each case remained in the piece that was to regenerate. The worms were cut as follows:

1. The ganglia were removed with as much of the tissue anterior to them as was necessary to make the cut, leaving however parts of the anterior end of the worm on both sides of the cut (Fig. A, 1).

2. The ganglia were removed from the side, with as little as possible of the lateral tissue and leaving all of the anterior tissue (Fig. A, 2).

3. The ganglia were removed in a right-angled piece, leaving the lateral part of the anterior end on one side (Fig. A, 3).

4. The ganglia were cut out with a straw, leaving a round hole in the worm (Fig. A, 4).

5a. The worm was cut in half longitudinally, and the ganglia removed from the pieces, or the ganglia were first removed with a straw, and the worm then cut (Fig. A, 5a). 5b. A slight variation of 5a was made by removing the ganglia with a straw, and a few days later, when the wound had closed, cutting the worm longitudinally (Fig. A, 5b). 5c. The anterior half of one side was cut off, and the ganglion of the other side cut out (Fig. A, 5c).

In all of these cases, unless as in a few instances the wound healed in such a way as to prevent growth of new tissue, regeneration took place, and externally, the worm appeared to be rapidly attaining the normal condition. The worms were not always kept until the form was completly restored, but regenera-

tion in Leptoplana: Anterior and Lateral Regeneration," *Jour. of Exp. Zool.*, Vol. I., p. 513, 1904. Among other results, he has found that anterior regeneration is complete when not much more than half of the cephalic ganglion is removed by a cross-cut.

tion was in every case rapidly taking place, and where checks were possible, pieces with and without ganglia were in the same condition. For example, in 5a, half-worms retaining one ganglion,

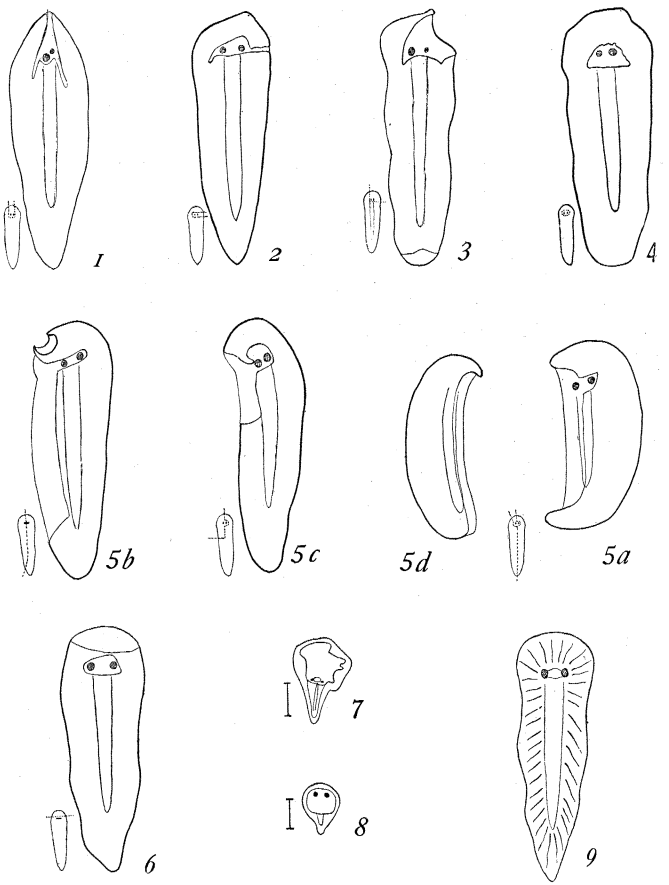

FIG. A, Diagrammatic figures of (1–6), worms regenerated after injury, indicated in each case by the accompanying small diagram. (7, 8), Regenerated pieces cut out of the head of the worm, and including cephalic ganglia and eye-spots. (9), Normal worm.

kept in the same dish with those without ganglia, regenerated at about the same rate.

In the pieces deprived of ganglia, the cut surfaces fused together, new tissue was formed, and eye-spots appeared in

about two or three weeks, often in two symmetrical groups, but sometimes with irregularities in size and position. The pieces at first were sluggish, and behaved like pieces from which the whole anterior end including the ganglia had been cut off, but as the eye-spots and other missing parts appeared the worms behaved more like the normal animal. In the light of what follows, it would be interesting to make more detailed observations on this point, for, in spite of the external signs of complete regeneration, sections show that the ganglia themselves have not regenerated. In the normal worm, the cephalic ganglia are well defined, and enclosed in a distinct sheath, and have the appearance in section of a brain of two hemispheres. The large nerve cords pass through the sheath and are connected with the ganglion (Fig. B, 1). In the regenerated worms, in place of the definitely defined cephalic ganglia, nerve fibers alone are present,

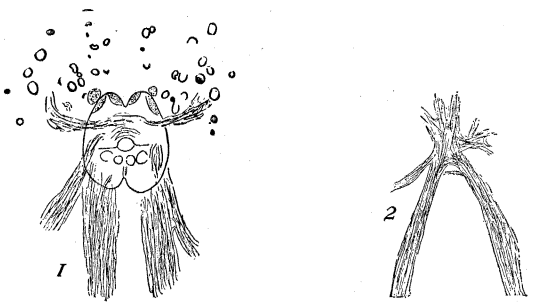

FIG. B (1), Diagrammatic figure of the cephalic ganglia, nerves and eye-spots of a normal worm, taken from a horizontal section. (2), Diagrammatic figure of the nerves in the position of the ganglia, in the regenerated worm of Fig. A (2), seven weeks after the removal of the cephalic ganglia.

which connect the large anterior and posterior nerve cords of the two sides of the body (Fig. B, 2). The eye-spots of the normal worm are more numerous and cover a larger area than in the regenerated worm. In the latter, the eye-spots are usually found in two well defined groups, though sometimes, crowded out of place or irregular, when the wound has healed in such a way as to leave too small a space for them to develop in the normal position.

In the absence of the ganglia it seems to be a fact that the presence of some anterior tissue is conducive to regeneration. It

will be noted that in all the cases described where regeneration occurred without the ganglia, some of the old tissue anterior to the level of the ganglia was left in the piece. The difference in regeneration with and without some anterior tissue was strikingly shown in the following experiment: the cephalic ganglia were removed with a straw, and the worm then cut in half longitudinally, the left half through an accident was injured, and some of the anterior tissue broken off. After seven weeks, the right half had regenerated well, and had for two weeks shown two distinct eye-spots, as in numbers of other similar cases. The left side, kept in the same dish for the same length of time, showed less regeneration, and no eye-spots had appeared, nor could any be found in sections (Fig. A, 5*a*, 5*d*). In all the cases where regeneration occurred in the absence of the ganglia, the cuts were made in such a way that lateral regeneration without anterior regeneration might account for the restoration of the form of the worm.

Regeneration of the anterior tip of the worm, that is when the worm has been cut off anterior to the ganglia, occurs in the absence of the ganglia as well as when they are present. The ganglia of several worms were removed with a straw, and after a few days, when the wound had closed, the anterior end was cut off anterior to the scar. Regeneration occurred as in check experiments, where the ganglia were retained, and by the time the eye-spots had reappeared, the form of the worm was completed (Fig. A, 6).

Summary and Conclusions.

The experiments show that:

1. Pieces containing the cephalic ganglia behave like normal animals, and regenerate readily.

2. Pieces deprived of the ganglia are sluggish, and may or may not partially regenerate.

3. Pieces cut across at any level posterior to the ganglia, do not regenerate to any extent anteriorly; but

4. Pieces deprived of the ganglia in such a way that some tissue anterior to the level of the ganglia remains in the piece,

regenerate partially. The normal form and behavior of the worm are restored, and the eye-spots are formed; but

5. The cephalic ganglia themselves never regenerate if completely removed.

6. Regeneration of the anterior tip of the worm occurs in the absence of the ganglia.

7. Lateral regeneration occurs in the absence of the ganglia, even though the ganglia themselves are not restored. It still remains unexplained why in the polyclads the tissue posterior to the cephalic ganglia does not regenerate the missing parts anterior to their level, and why the tissue around the ganglia does not restore them. It seems clear that the absence of the ganglia is in some way directly responsible for the lack of anterior regeneration, for when the ganglia are not entirely removed, complete regeneration occurs. The completion of the form of the worm and regeneration of eye-spots (though without the ganglia) in experiments 1–5 may have come about mostly through lateral regeneration, for in each case anterior tissue remained on one or both sides.

My work was carried out at the Marine Laboratory of Leland Stanford University. It gives me pleasure to express my appreciation of the courtesy extended to me while at Pacific Grove.

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